An Overview of Surface Heat Microbial Reduction as a Viable Microbial Reduction Modality for Spacecraft Surfaces

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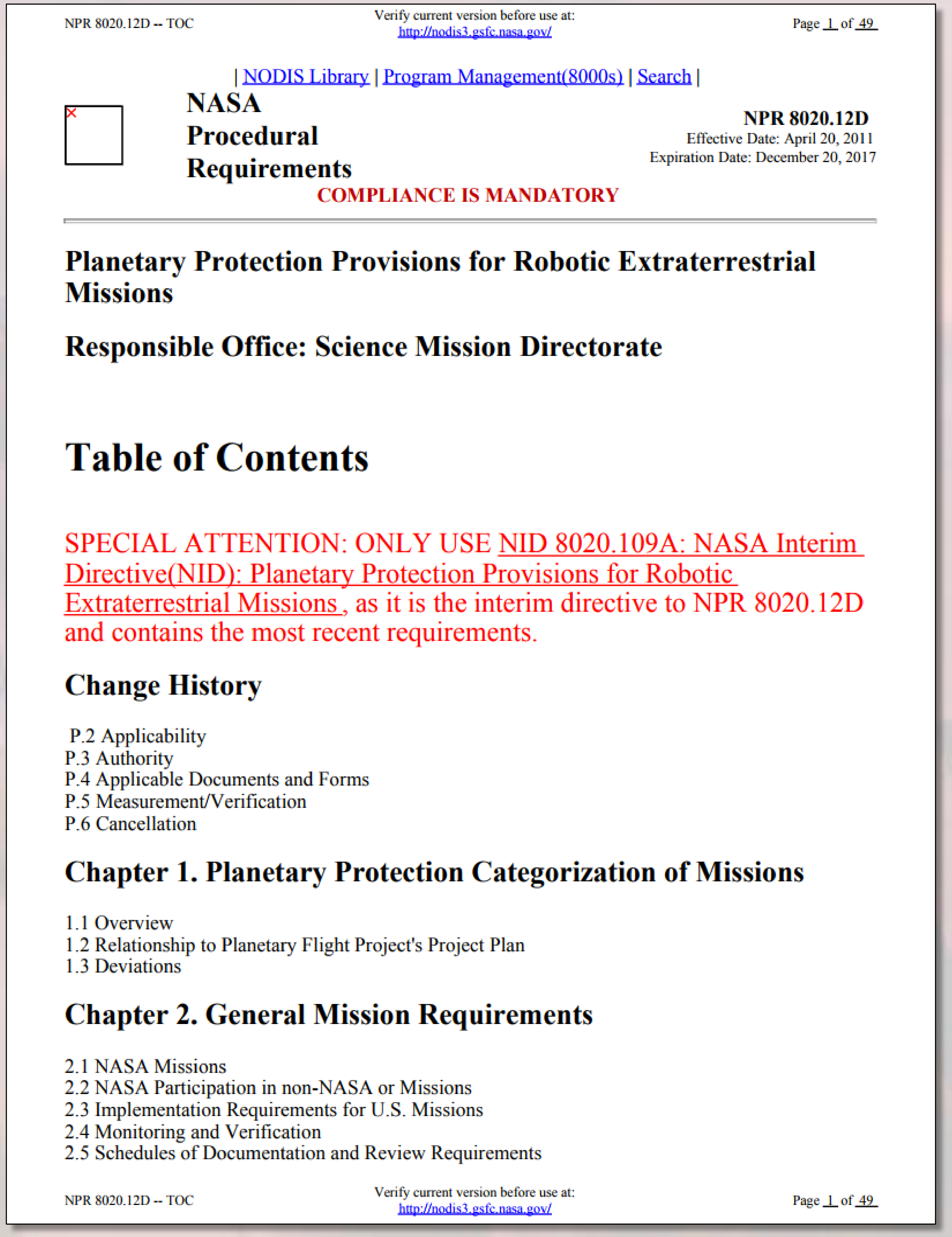
NASA Planetary Protection Policy

- NASA Planetary Protection (PP) policy establishes the microbial reduction requirements for flight project hardware to prevent the forward contamination of planetary bodies with Earth organisms.
- NASA PP policy is set forth in NASA Procedural Requirement 8020.12D (NID 8020.109A), Planetary Protection Provisions for Robotic Extraterrestrial Missions.



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NASA
Procedural
Requirements

NPR 8020.12D
Effective Date: April 20, 2011
Expiration Date: December 20, 2017

COMPLIANCE IS MANDATORY

Planetary Protection Provisions for Robotic Extraterrestrial Missions

Responsible Office: Science Mission Directorate

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SPECIAL ATTENTION: ONLY USE NID 8020.109A: NASA Interim Directive(NID): Planetary Protection Provisions for Robotic Extraterrestrial Missions, as it is the interim directive to NPR 8020.12D and contains the most recent requirements.

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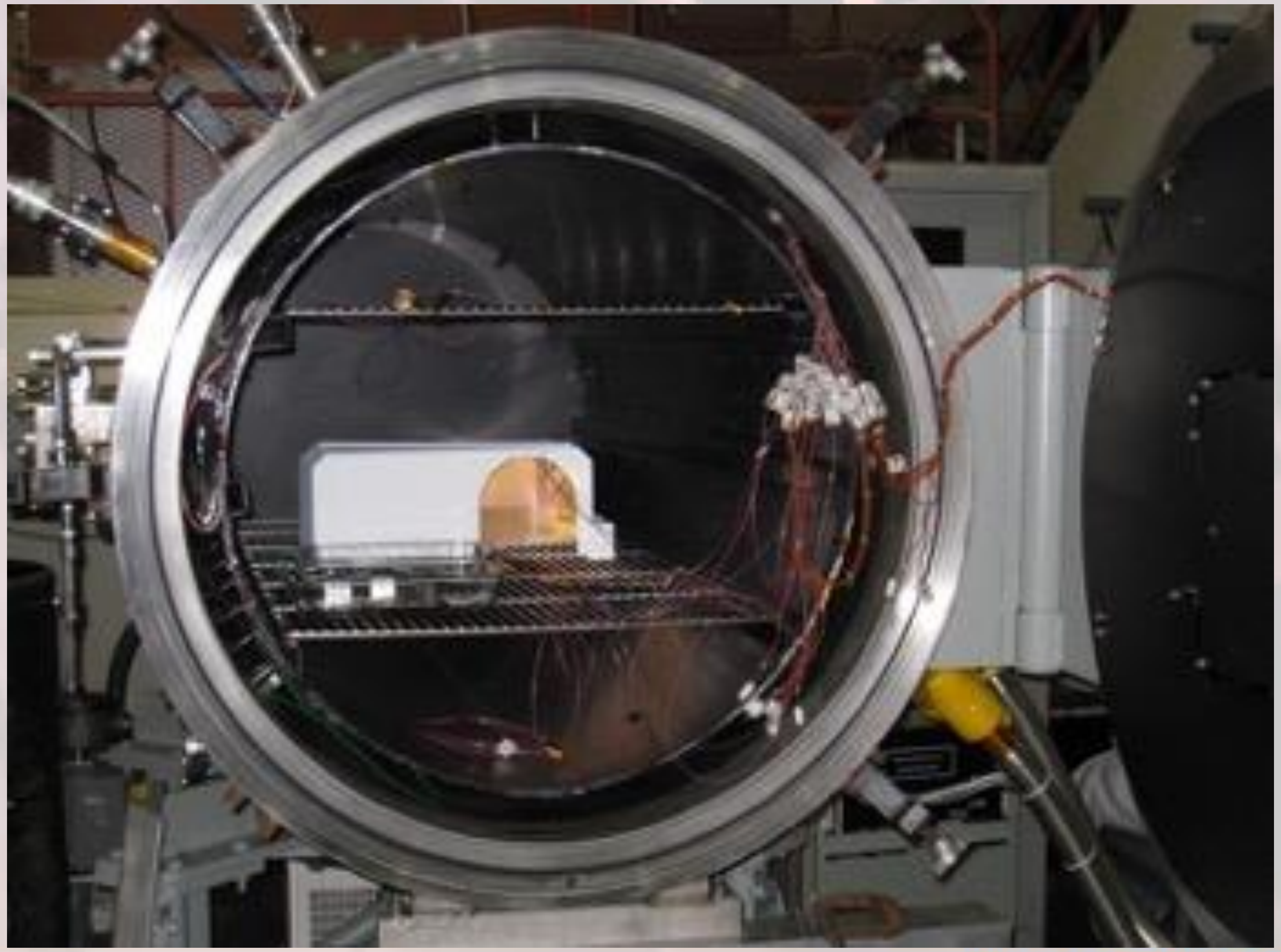
NASA Planetary Protection Policy

- Of the approved microbial reduction processes available for flight project implementation, dry heat microbial reduction (DHMR) is the process most often employed by the Jet Propulsion Laboratory Biotechnology and Planetary Protection Group (JPL BPPG) to ensure compliance with NASA policy on flight hardware biological cleanliness.
- HMR is employed on assemblies with large surface areas, difficult to clean, or with limited access, and on assemblies with encapsulated bioburden in bulk materials
  - Honeycomb Structure
  - Multi-layer Insulation (MLI)
  - Backshell/Heat Shield
  - Cabling
  - Electronic Components

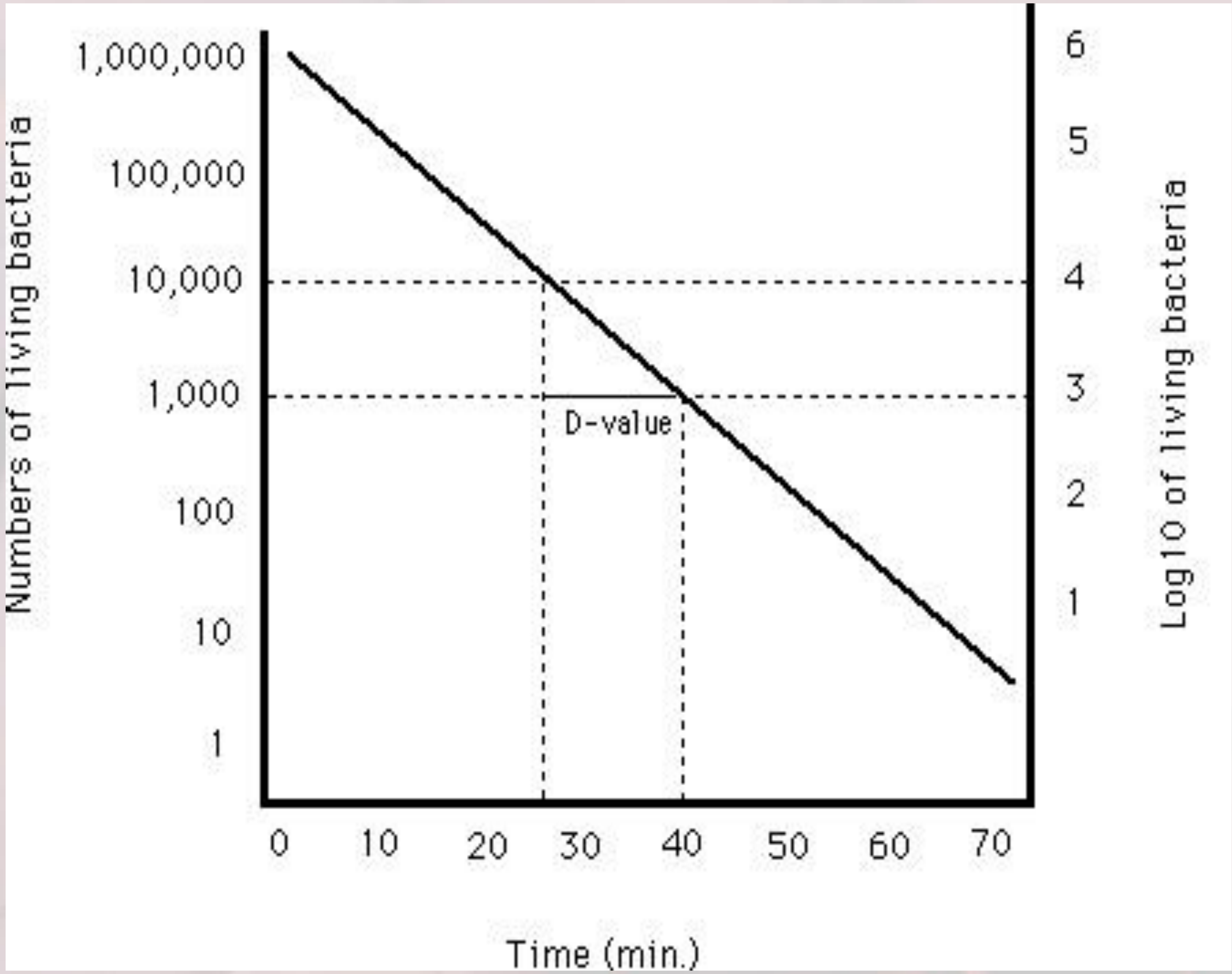


Heat microbial reduction background

- In 2013, NASA revised the longstanding DHMR specifications outlined in NPR 8020.12D in order to integrate the latest findings on heat microbial reduction (HMR) processes.
- With support from the Mars Program Office, the revisions were based in large part on experimental results generated by the JPL BPPG in conjunction with the European Space Agency, which in turn were used to provide recommendations for the revised NASA specifications
- Notable changes to DHMR specifications include:
    - ”Dry” (< 25% relative humidity at 0 °C and 1 atmosphere) heat no longer a strict requirement
        - Alternate measureable definitions: 1.15 torr water vapor partial pressure or a dew point of less than –16°C.
    - Allowances for HMR processes that occur under “ambient” (controlled to 70% RH at 20 °C under 1 atmosphere) humidity conditions.
    - Applies to exposed and mated surfaces
    - No humidity requirement for encapsulated spores

What are D-values?

- Decimal reduction value
- If a homogenous suspension of microorganisms is heated at a constant temperature, the microorganism destruction commonly follows logarithmic order or destruction/death
- D-values are the time required to reduce the overall bacterial population 10-fold or 90% at a specific temperature.
- Does not depend upon the initial number of microorganisms present in the suspension



Changing paradigms

Old paradigm (MSL-era and prior)

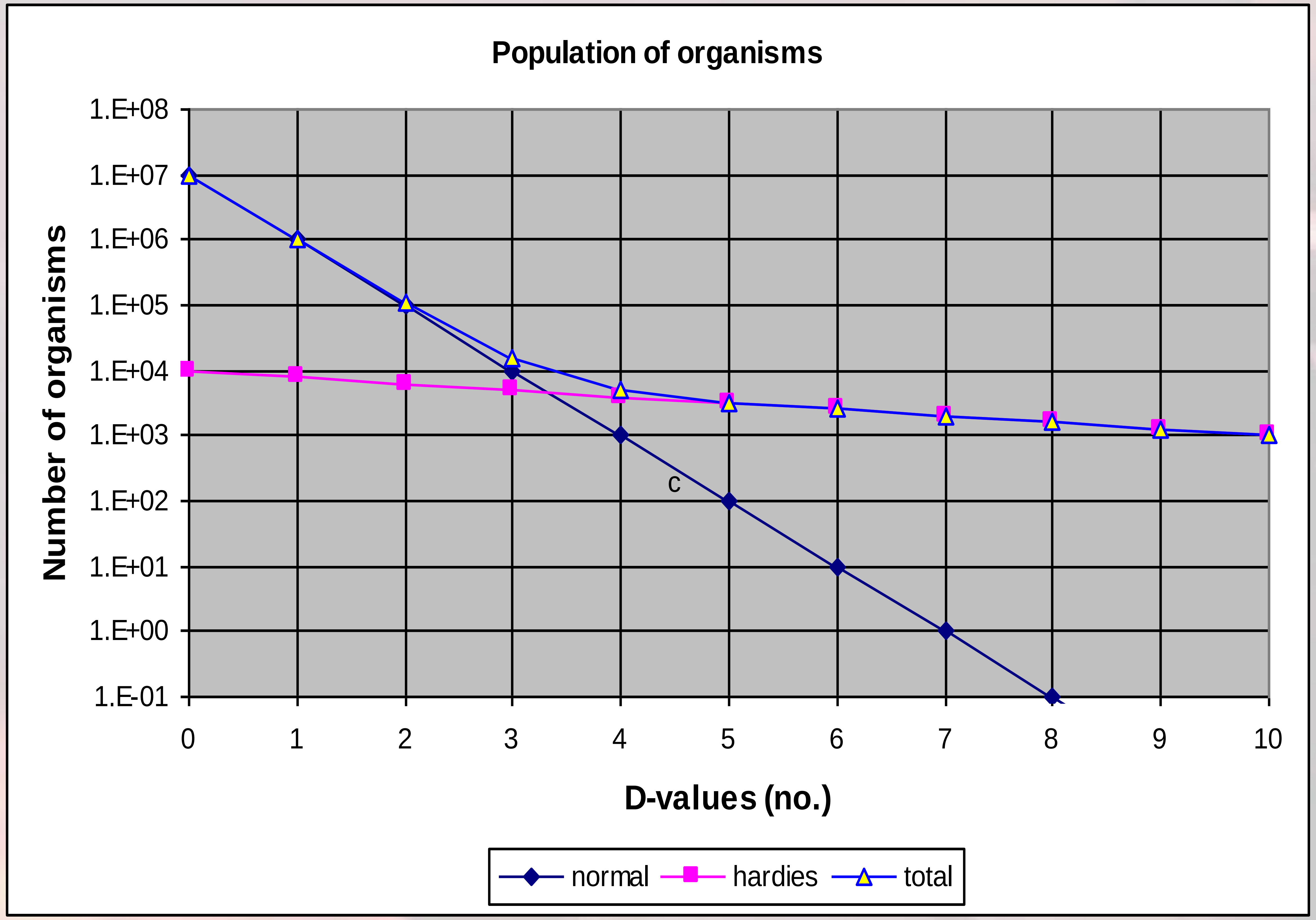
- Applicable within the temperature range of 104 ºC to 125 ºC
- Bioburden reduction credit limited to a 1 to 3-log reduction
- D-value (D_{125}) for 1-log reduction
    - 0.5 hrs. for exposed surfaces
    - 1.0 hrs. for mated surfaces
    - 5.0 hrs. for encapsulated surfaces

New Paradigm (Mars 2020 and InSight)

- Applicable within the temperature range of 110 ºC to 200 ºC
- Bioburden reduction credit range from 2 to 6-log reduction
- For **mated** surfaces, the *D*-value is twice the *D*-value for free surfaces
- For **encapsulated**, the *D*-value is ~5 times the *D*-value for a free surface
- A 25% margin added to the D-value for Mars 2020 and InSight
- Limited to 4-log reduction for process temperatures 110 ºC to 125 ºC

Time-temp requirement based on lethality curves
developed from empirical data generated under peer-vetted methods in parallel at JPL and ESA

Log-reduction “credit” is limited by the population of heat-resistant organisms



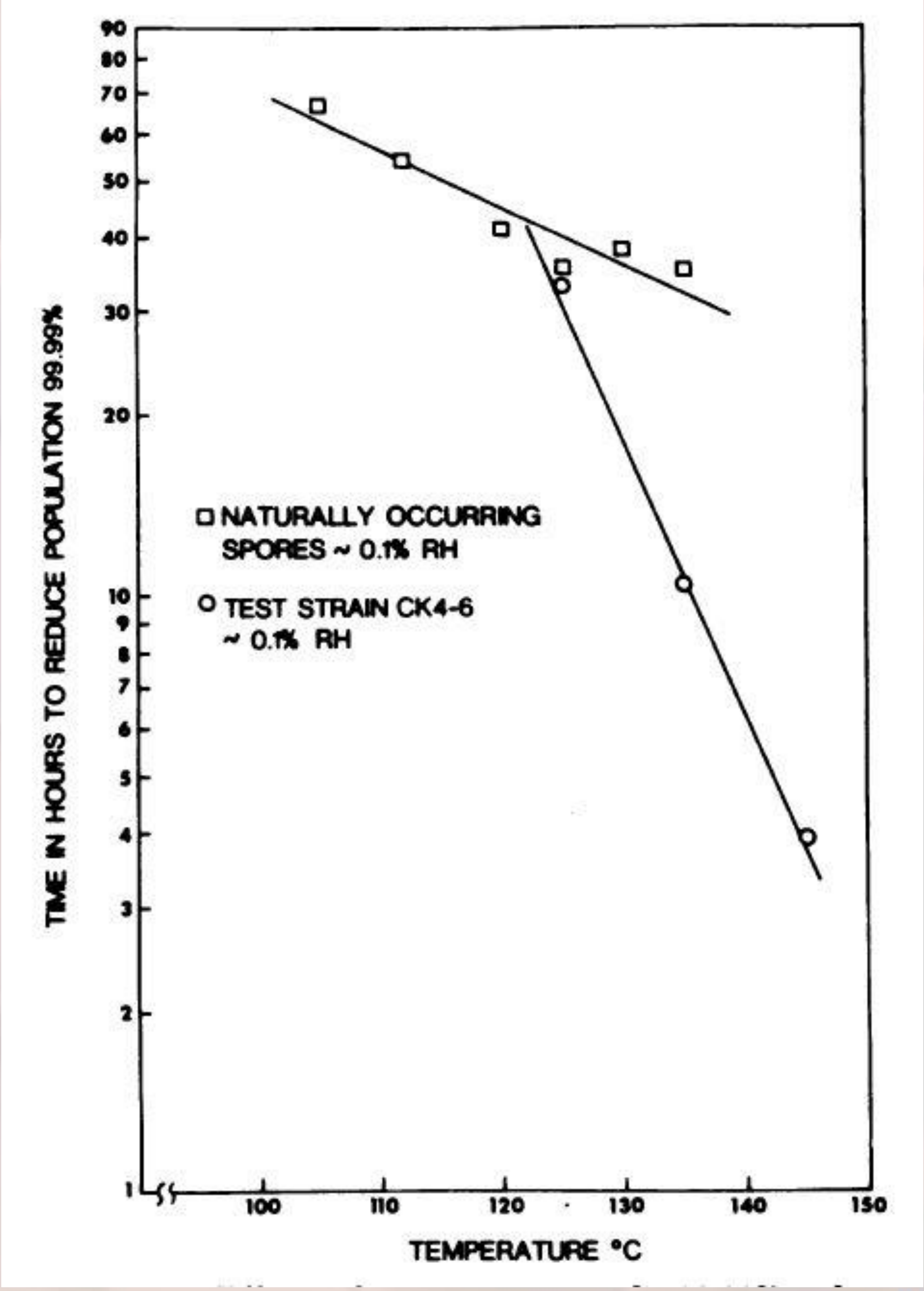
Some of the population is easily inactivated, but the “kill-rates” cross and the total population does not decrease faster than the more resistant organisms.

Potential benefits of current specifications

- Allow routine bioburden reduction credit for heat treatments of hardware at temperatures up to 200 °C, whether or not the process was performed for PP purposes, or whether it was performed under controlled or ambient humidity conditions.
- Simplify the requirement and reduce the cost for DHMR by allowing use of ambient humidity ovens instead of vacuum ovens. Projects could get credit for heat treatments without having to use controlled humidity environments.
- Reduce mission costs associated with not having to reach 500 °C for 0.5 seconds before bioburden reduction credit can be obtained for atmospheric entry heating in break up and burn up analyses.
- Increase the bioburden reduction credit beyond the 4-log reduction limit for extended DHMR heating times.
- Facilitate spacecraft hardware manufacturing to achieve sterility (accounting “zero” survivor organisms).

Evidence for additional hardy spores in the environment

- Evidence of resistant spores in a mixed natural population. This natural population is an uncharacterized mixed group of organisms.
- A spore preparation of ATCC 29669, is representative of a population subset showing great resistance.
- This has been a consideration in establishing our current specifications.



Revised/current JPL HMR specifications

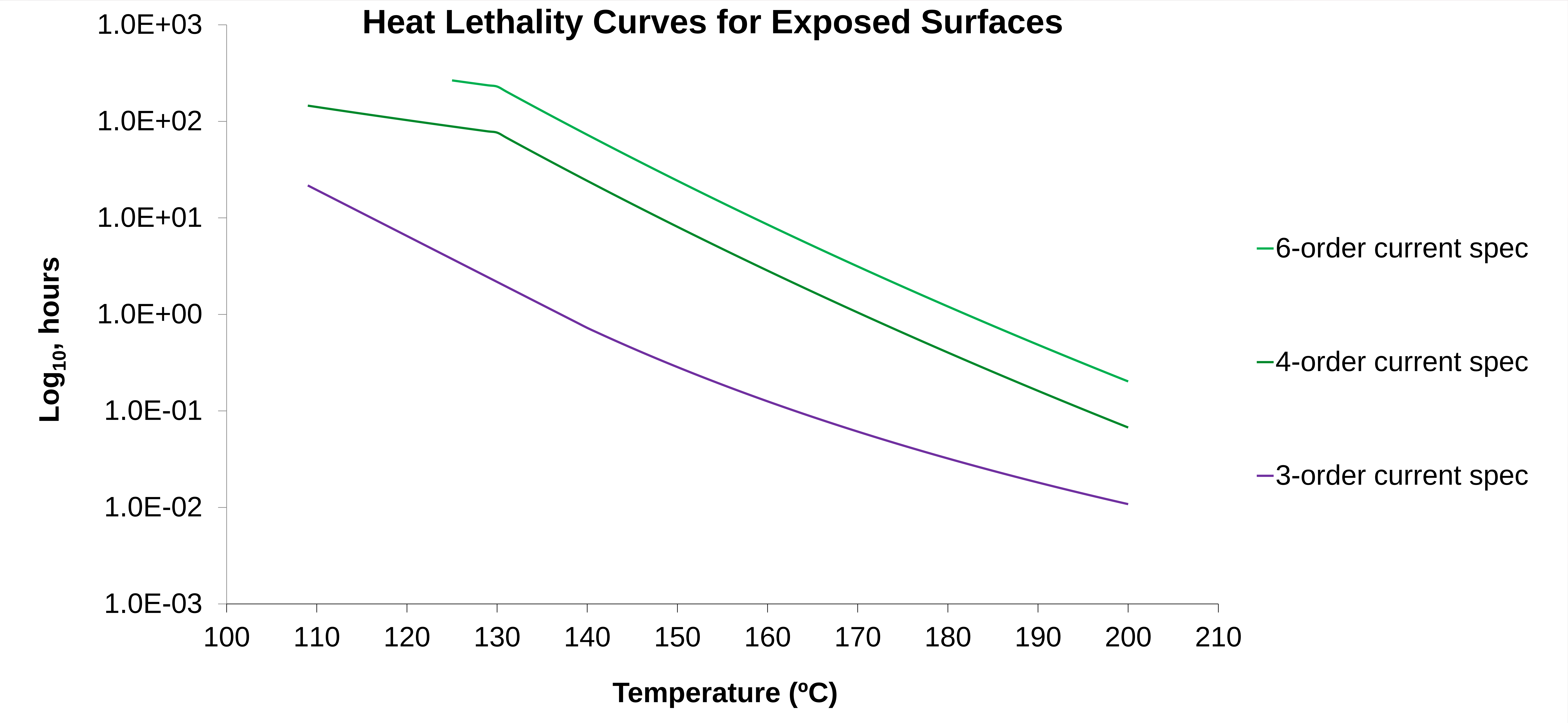
Heat microbial reduction D-values for reducing spore population by 4 to 6 orders of magnitude.

Configuration	D-Value, hours	Humidity	Temperature, T, °C
Surfaces	$0.5 * 10^{((125 – T) / 21)}$	Dry	$110 \le T \le 140$
Surfaces	$0.0965 * 10^{((140 – T) / (23 * T / 140))}$	Dry	$T > 140$
Surfaces	$0.0965 * 10^{((140 – T) /18))}$	Ambient	$110 \le T \le 140$
Surfaces	$0.0965 * 10^{((140 – T) / (23 * T / 140))}$	Ambient	$T > 140$
Surfaces	$10 * 0.5 * 10^{((125 – T) / 21)}$	Uncontrolled	$110 \le T \le 140$
Surfaces	$10 * 0.0965 * 10^{((140 – T) / (23 * T / 140))}$	Uncontrolled	$T > 140$
Encapsulated	$5 * 0.5 * 10^{((125 – T) / 15)}$	Uncontrolled	$116 \le T \le 125$
Encapsulated	$5 * 0.5 * 10^{((125 – T) / 21)}$	Uncontrolled	$125 \le T \le 140$
Encapsulated	$5 * 0.0965 * 10^{((140 – T) / (23 * T / 140))}$	Uncontrolled	$T > 140$

Heat microbial reduction D-values for reducing spore population by 4 to 6 orders of magnitude.

Configuration	D-Value, hours	Temperature, °C
Surfaces, mated, and encapsulated	$10^{(–3.5991 + 2049.0923 / (T + 273))}$	$110 \le T \le 130$
Surfaces, mated, and encapsulated	$10^{(–19.1595 + 8320.082 / (T + 273))}$	$T > 130$

Revised/current JPL surface specifications



HMR Background

Foundational Studies

- Bigelow, 1921. The Logarithmic Nature of Thermal Death Time Curves. JID.
- Etsy and Meyer, 1922. The heat resistance of the spores of B. botulinus and allied anaerobes. JID.

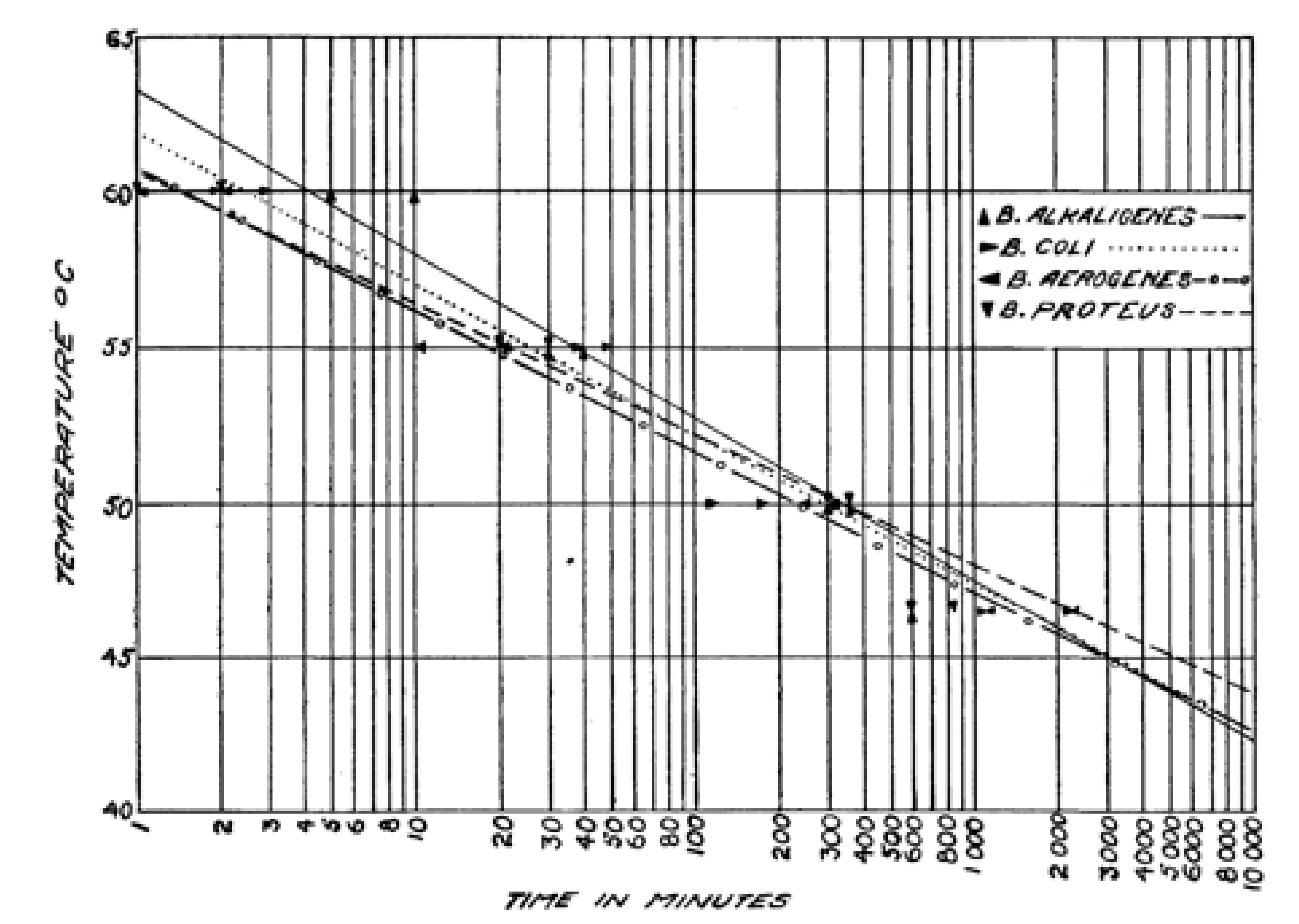


Chart 5.—Thermal death time curves of four non-spore-bearing organisms.

Bigelow, 1921

Linear-Bigelow Equation (Mafart and Leguer, 1998)

$DT = D_T * 10^{-(1/z)(T-T^*)}$

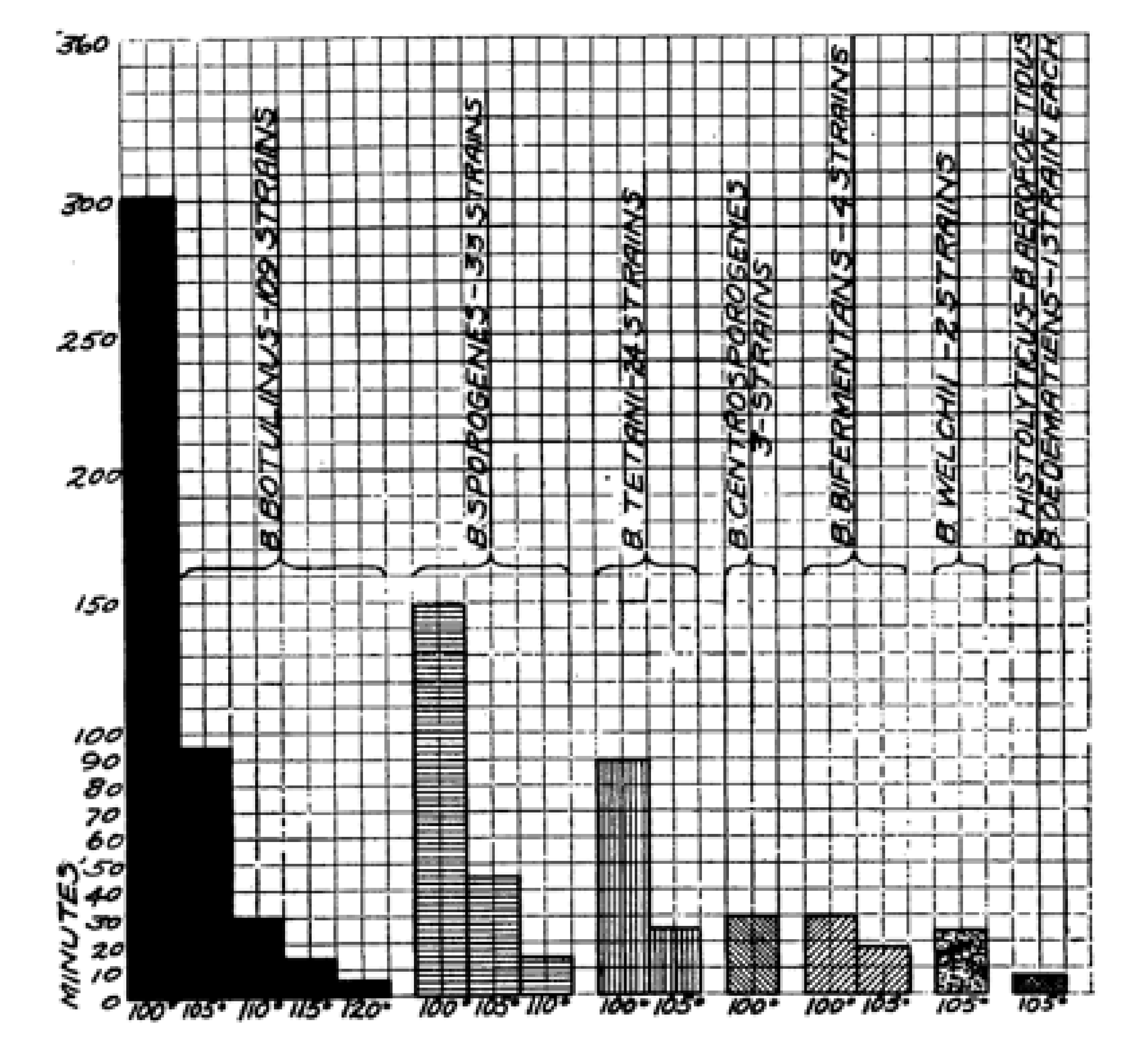


Chart 1.—Maximum heat resistance of sporulating anaerobes.

Etsy and Meyer, 1922

First-order reaction for microbial death kinetics (Mafart and Leguer, 1998)

$N = N_0 e^{-kt}$

Reference data and biological indicators

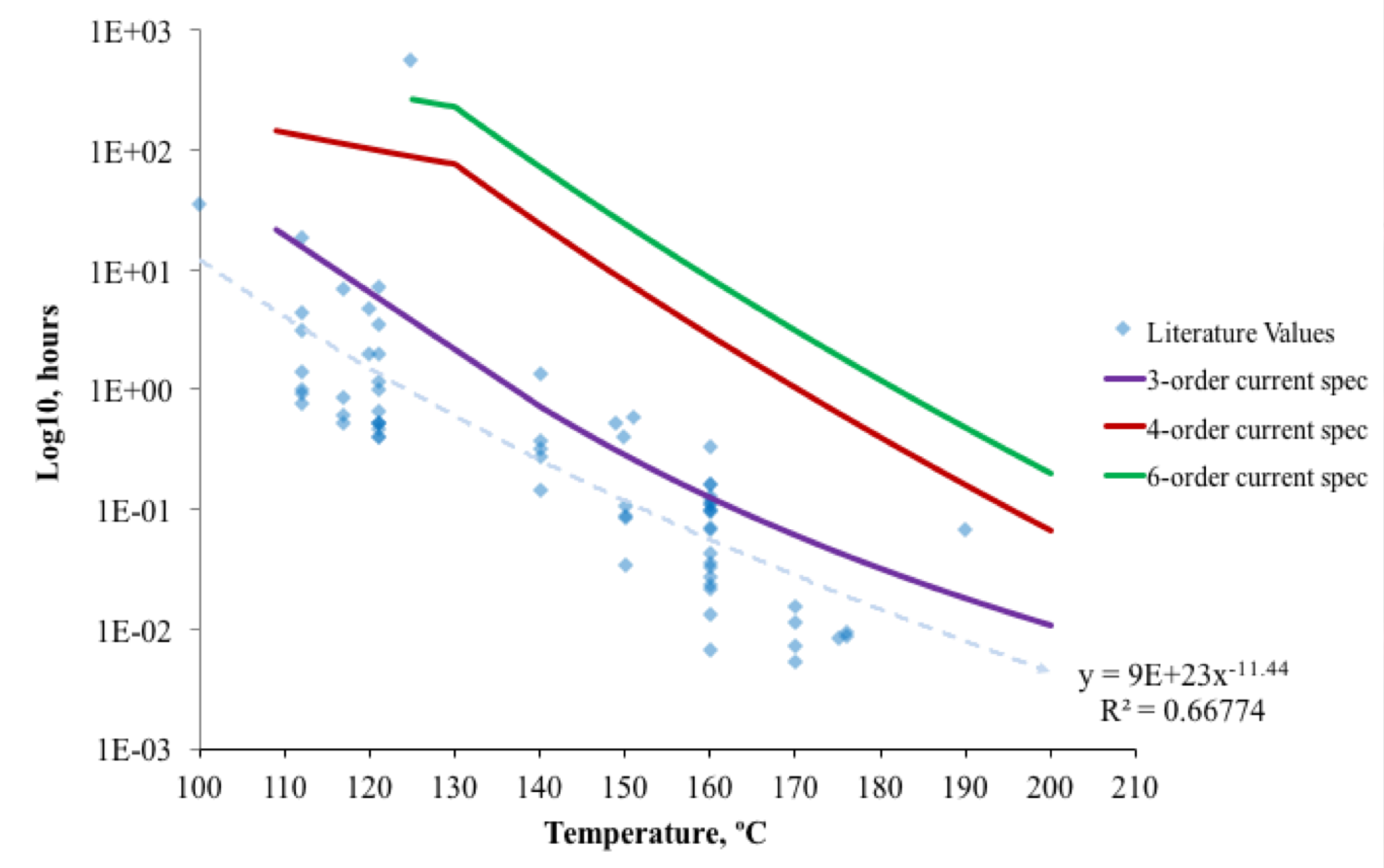
Literature
- Includes *Bacillus* spp. and *Clostridium* spp.
- Data derived from published literature

B. atrophaeus – JPL
- Strain ATCC 9372
- Original B.a. strain investigated by JPL
- Used here and in industry as a biological indicator for standard (non-hardy) spores
- Data derived from JPL studies
- Studies also performed by ESA using the same ATCC 9372 strain investigated by JPL
- Data derived from ESA studies in parallel with JPL

ATCC 29669 - JPL
- Atypical, hardy (heat-resistant) *Bacillus* sp.
- Spores of this strain exhibits the highest heat resistance of cleanroom-associated bacteria
- Data derived from JPL studies

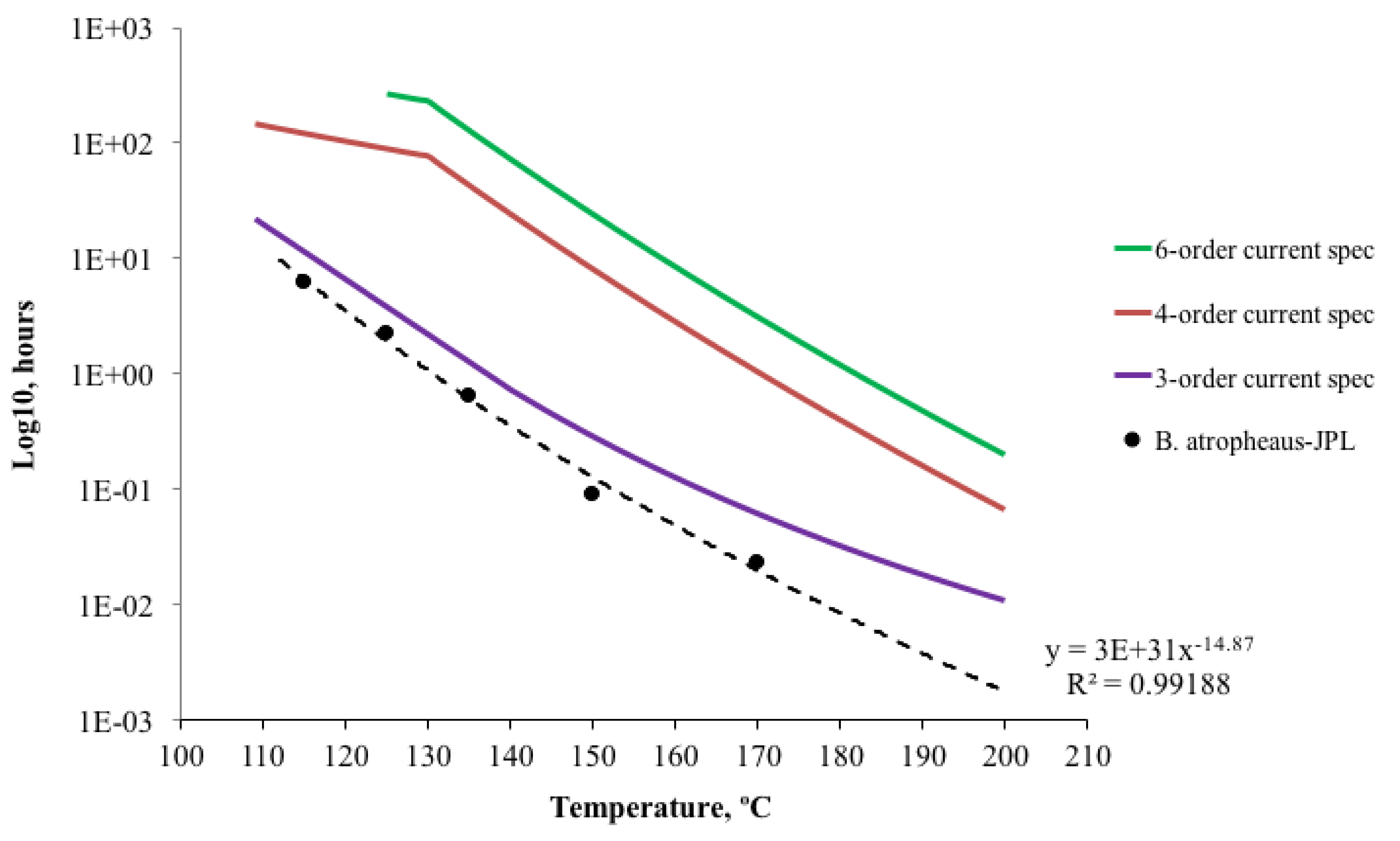
Published literature data for DHMR

Reference data vs. Mars 2020 HMR for 3-,4-, and 6-log reduction +25% margin



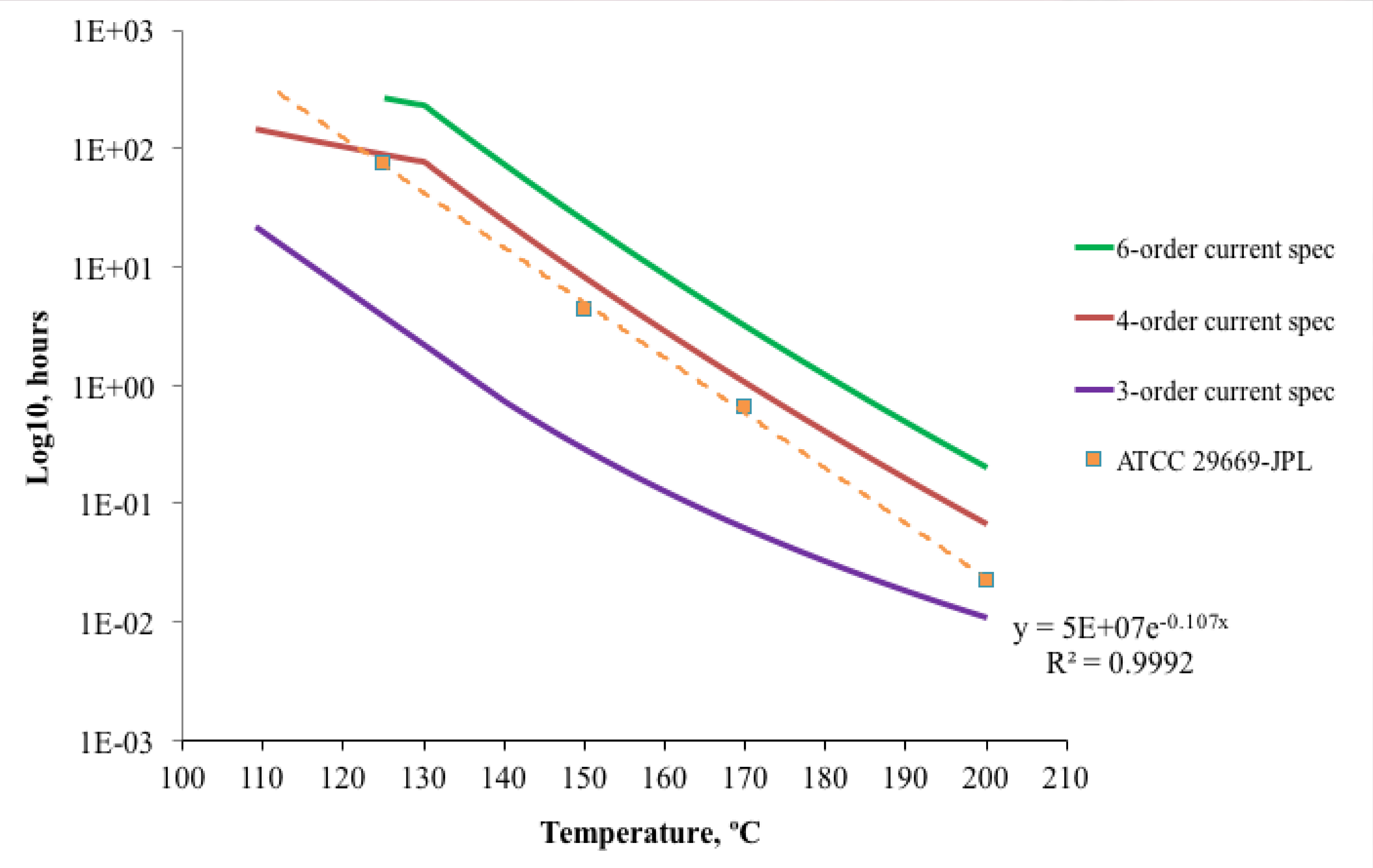
B. atrophaeus - JPL reference strain

4-log reference strain vs. Mars 2020 HMR for 3-,4-, and 6-log reduction +25% margin



ATCC 29669 - JPL reference strain

4-log reference strain vs. Mars 2020 HMR for 3-,4-, and 6-log reduction +25% margin



HMR Log margins

3-, 4- and 6-log reduction

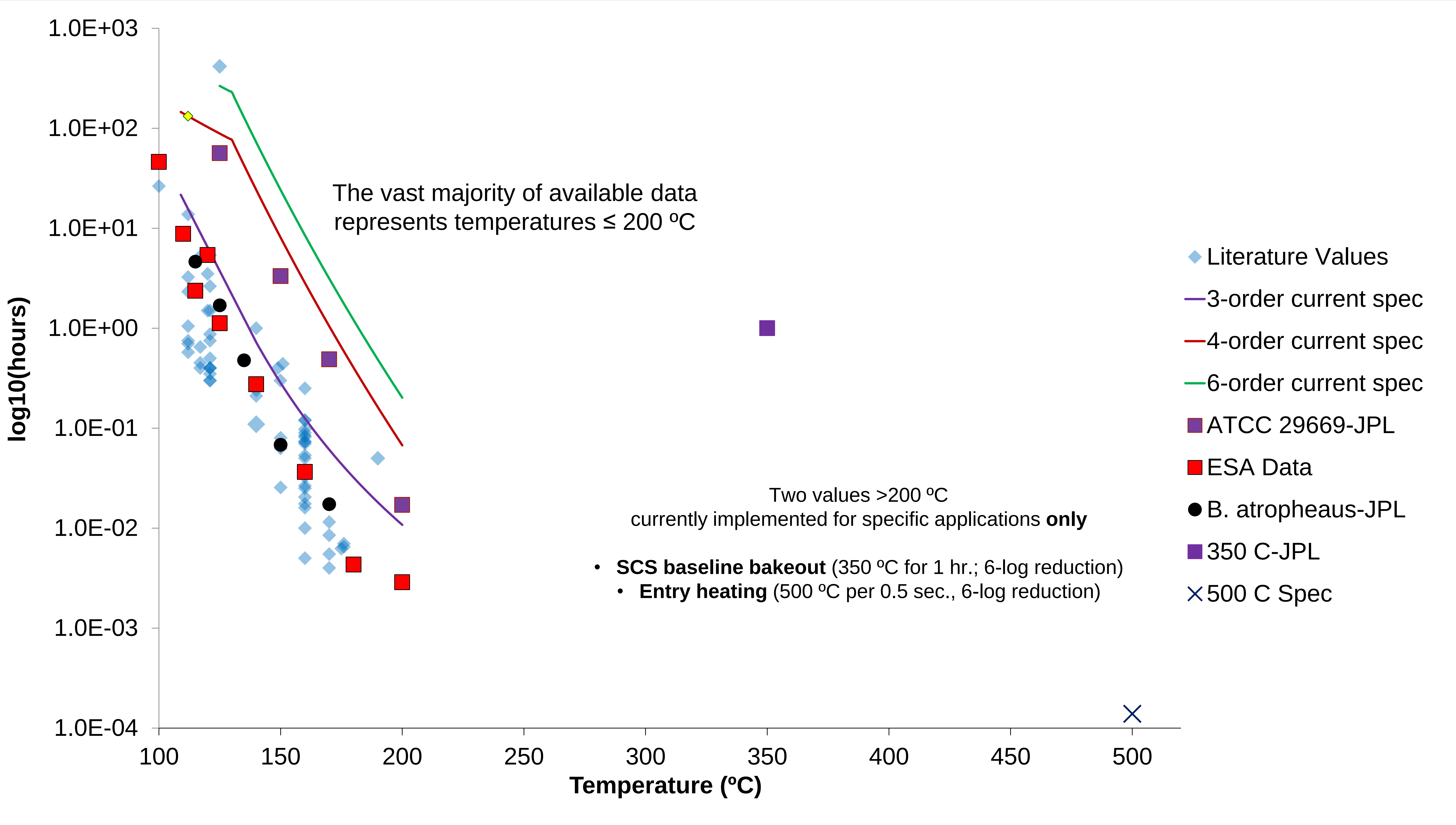
3-log	$\triangle \log_{10}(\text{time}) = \log_{10} \frac{Time_{Ref}}{Time_{Spec}}$				
	112 ºC	**125 ºC**	**150 ºC**	**170 ºC**	**200 ºC**
Literature	-0.83	-0.68	-0.26	-0.49	-0.81
***B. atropheaus*-JPL**	-0.36	-0.35	-0.44	-0.55	-0.95
ATCC 29669-JPL	+1.20	+1.18	+1.25	+0.90	+0.20

4-log*	$\triangle \log_{10}(\text{time}) = \log_{10} \frac{Time_{Ref}}{Time_{Spec}}$				
	112 ºC	**125 ºC**	**150 ºC**	**170 ºC**	**200 ºC**
Literature	-1.64	-1.93	-1.55	-1.60	-1.48
***B. atropheaus*-JPL**	-1.12	-1.59	-1.72	-1.66	-1.57
ATCC 29669-JPL	+0.37	-0.07	-0.03	-0.21	-0.47

6-log	$\triangle \log_{10}(\text{time}) = \log_{10} \frac{Time_{Ref}}{Time_{Spec}}$				
	112 ºC	**125 ºC**	**150 ºC**	**170 ºC**	**200 ºC**
Literature	-	-	-2.08	-1.90	-1.78
***B. atropheaus*-JPL**	-	-	-2.25	-1.96	-1.92
ATCC 29669-JPL	-	-	-0.56	-0.51	-0.77

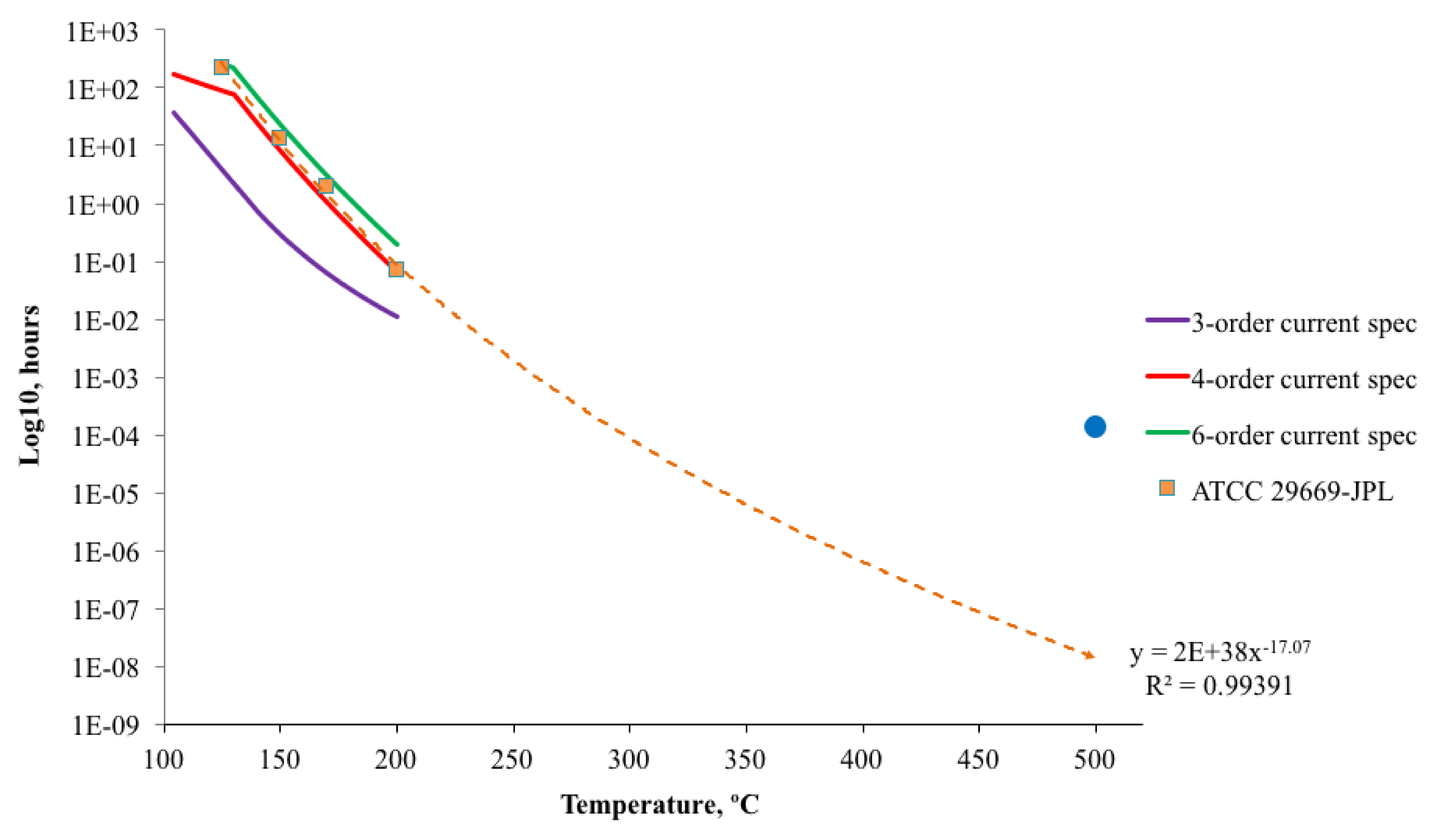
- Reference data compiled from log:log comparisons to the revised spec values calculated for ambient conditions
- Positive/Negative values: Reference value above/below the spec value. Denotes conservatism in the spec where negative

HMR Snapshot



ATCC 29669 projection >200 ºC

12-log projection



Summary

- The MSL-era requirements were an improvement over the Viking-era DHMR practices, but still did not account for the hardy spore population
- The newly revised requirements for HMR were developed to account for non-hardy *and* hardy spores…

…thus, the newly revised spec values for HMR are more stringent than the previous requirements

- The findings reported here indicate the revised NASA HMR specifications from 110 °C to 200 °C are appropriate for achieving 4-log and 6-log reductions with hardy spore populations
- For non-hardy spore populations, or for temperatures above 200 °C, the specifications are exceedingly conservative
- For more detailed information regarding HMR at temperatures above 200 °C, additional studies are necessary

Resources

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